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In Vivo Changes in the Activity of Gill ATPases, Muscle Cell Volume, and Plasma and Cellular Ionic Concentration of the African Catfish, *Clarias gariepinus* Induced by Atrazine or DDT at Different Sublethal Concentrations

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ABSTRACT. The study concerns the effects of the herbicide atrazine and the insecticide p,p'-DDT at different sublethal doses and during various test periods on branchial Na^+, K^+, Mg^{2+}-ATPase activities, ionic composition of the plasma and muscle and the volume of muscular cells in the african catfish *Clarias gariepinus*. The most important significant changes in Na^+, K^+, ATPase activity during exposure to atrazine were represented by decrease at the lower concentration (100 μg/liter) and increase at the higher concentration (500 μg/liter). Exposure to 500 μg/liter DDT produced a short and strong activation of gill Na^+, K^+,- ATPase which was followed by recovery to controls level. While Mg^{2+}-ATPase was activated from exposure hr 72 at both atrazine concentrations, it was inhibited during the whole experimental time in DDT (24 hr). Sodium and potassium concentrations in plasma and muscle cells as well as cell volume were changed during exposure to both chemicals, which might indicate osmoregulation difficulties. The value of the measured responses as an indicator of stress caused by water contamination is discussed.

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Fish were held under these conditions for at least three weeks prior to experimentations. A photoperiod of 16 hr of light and 8 hr of dark was maintained, oxygen was never below 8 mg/liter, acidity was monitored regularly (pH 7.3 ± 0.4) using pH meter. Fish were fed on a cat fish diet (35% protein) at a rate of 3% body weight once every other day, feeding was interrupted 24 hr before the start of experiments and during their duration. Two days prior to the application of chemicals fish were transferred to a 20 liter glass test aquaria. The fish loading factor was 8 per aquarium.

Chemicals

Atrazine and DDT were obtained as technical grade from Riedel Ltd, and Stock solutions were prepared in ethanol. Toxicants were added to the glass aquaria in very small volumes of solvent. Controls contained equivalent volumes of solvent.

Experimental Design

The experiments were started by adding the ethanolic solution of atrazine or DDT to the water in test aquaria to have final sublethal concentrations of 1) 100 μg/liter atrazine, 2) 500 μg/liter atrazine, 3) 500 μg/liter DDT. The fish were sacrificed in groups of 8 after 3, 6, 9, 24, 48, 72 and 168 hr in each atrazine concentrations, and after 3, 6, 9 and 24 hr in the DDT test concentration. In a separate preexperiments, the sublethal doses were determined after the addition of the pollutants to the water in known quantities until a lethal dose was obtained (Atrazine 8000 μg/l, DDT 4000 μg/l). During exposure, water was changed completely every day.

Blood and Tissues Sampling

The fish were caught and rapidly anaesthetized in MS222 solution (50 ppm) in a separate aquarium. Immobilization was achieved within 20 sec. Blood was collected from a severed portion posterior to the head on the dorsal side. The plasma was obtained by centrifugation of the blood containing ammomium heparinized vials for 3 min at 3000 rpm and stored in a deep freezer. Individual gill arches were separated and gill filaments removed and frozen. After blood and gill sampling, the skin was removed and a piece of white expaxial muscle was taken from a definite area below the dorsal fin. It was weighed and dried to constant weight at 85°C for 48 hr and then reweighed.

Analytical Techniques

Plasma sodium was measured by flamephotometry using 5 μl plasma. Plasma potassium was determined by atomic absorption spectrophotometry using an air acetylene flame. Spectral interference between Na^+ and K^+ was minimized by adding 5% CsCl to both samples and standards. The inorganic solutes of muscle tissue were extracted from wet muscle tissue with 0.1N HNO_3 for 25 hr at 60°C. This procedure proved to be the most efficient of the several employed techniques (Assem and Hanke, 1979). No increase in ion values were found after extraction for a longer time. Na and K concentrations were again measured by flamephotometry with 5% CsCl added to avoid interference variation.

were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical Analysis

Two-way analysis of variance was done on the data obtained. Where significant differences were evident, differences between the means were assessed statistically using student's t-test. The results were demonstrated as percentage of changes vs controls (100%), and a triangle was added when the t-test of the original values (not the relative values) indicated that the means were statistically significant ($P < 0.05$).

Results

Effects on the Na^+-K^+ Dependent ATPase

The results of in vivo effects of atrazine on the gill enzyme specific activity are demonstrated in Fig. (1). While an inhibition of the enzyme activity was observed at the lower concentration (100 µg/liter), an activation was measured at the higher concentration. This reaction of the enzyme to the presence of atrazine was followed by a significant and positively increasing activation of the enzyme at both concentrations from exposure hr 24 and which lasted throughout the rest of the experimental time. Recovery to normal control levels after 168 hr was not recorded.

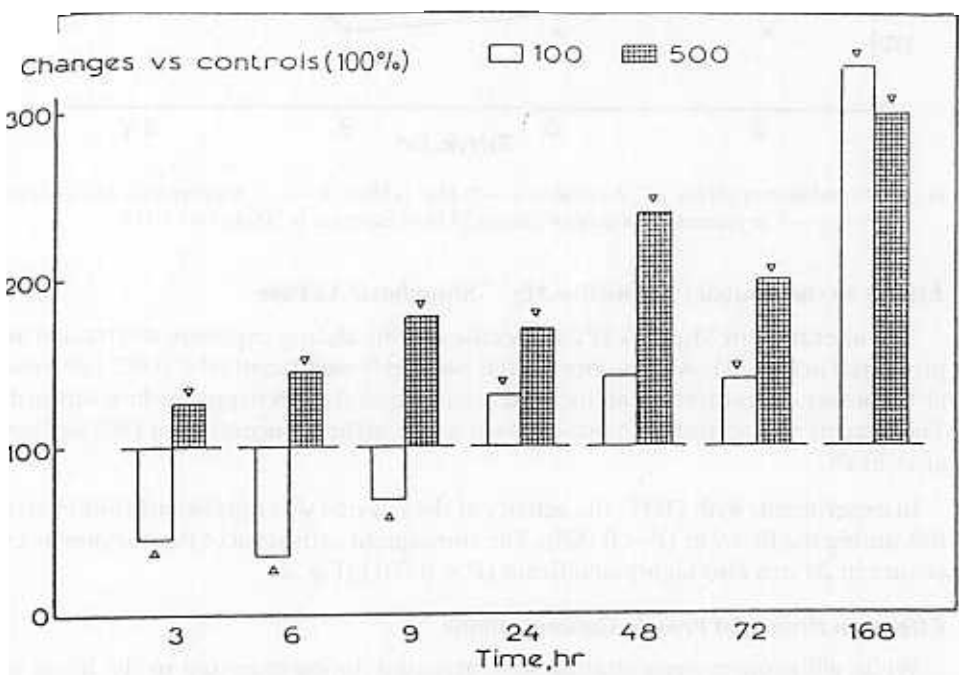
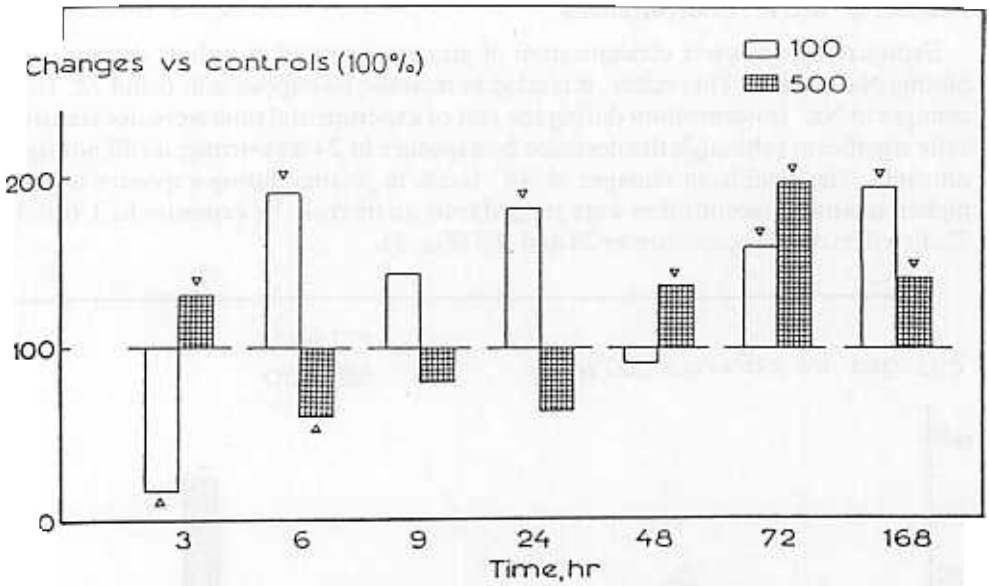


FIG. 1. Changes of gill Na^+-K^+ ATPase activity level in percentage of controls during 168 hr of exposure to 100, 500 µg/l atrazine.

of exposure. Recovery to normal controls level after 168 hr was not recorded at both atrazine concentrations (Fig. 4).



3. Changes of gill Mg^{++} ATPase level in percentage of controls during 168 hr of exposure to 100, 500 µg/liter atrazine.

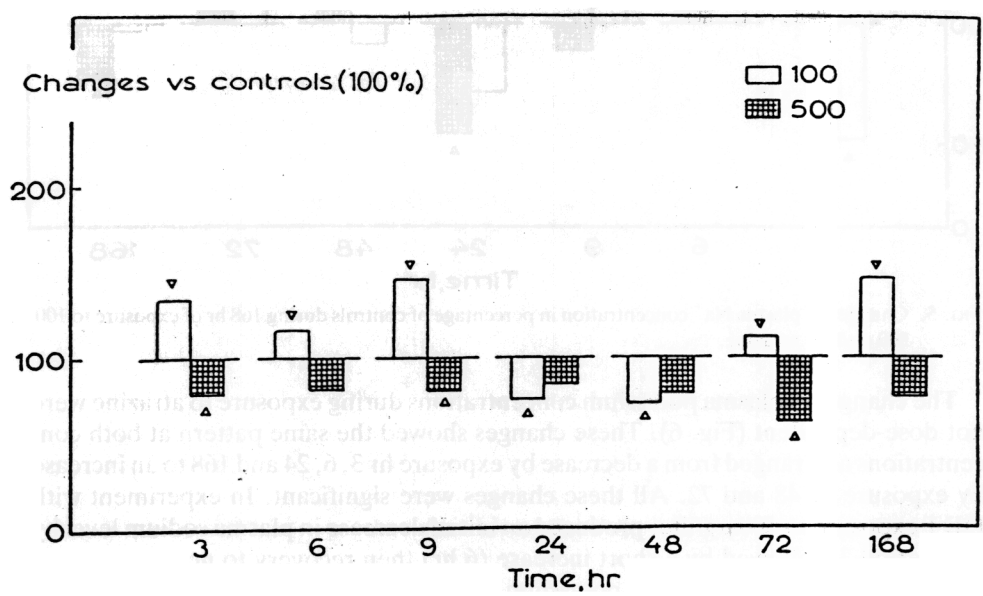


FIG. 4. Changes of gill protein content in percentage of controls during 168 hr of exposure to 100, 500 µg/liter atrazine.

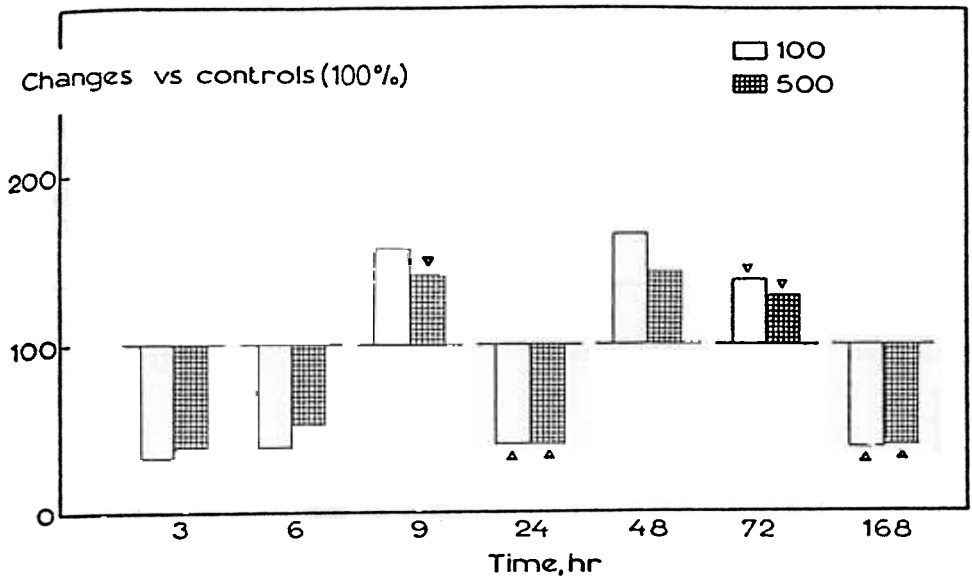


FIG. 6. Changes of plasma K^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

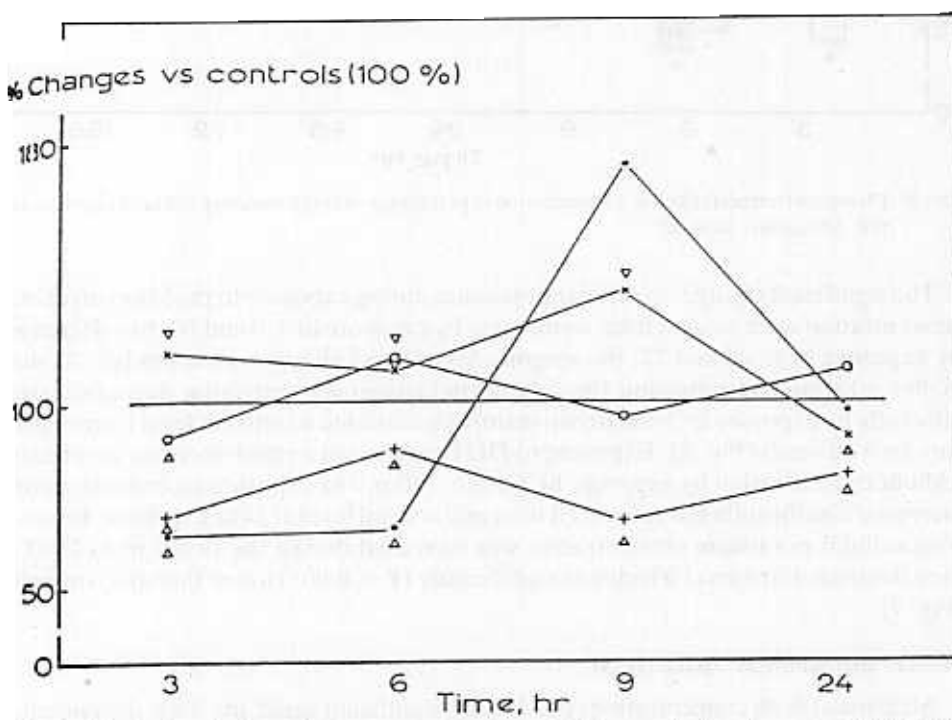


FIG. 7. Correlation of plasma Na^+ concentration ○——○, K^+ concentration +——+, muscle (ICS) Na^+ content •——• and muscle (ICS) K^+ content ×——× in percentage of controls during 24 hr of exposure to 500 μg/liter DDT.

lowed by cellular shrinkage from the 9th hr onward. Till the end of exposure time (168 hr), no indication of cell volume recovery was recorded (Fig. 10). In vivo exposure to DDT produced a highly significant ($P < 0.005$) cellular dehydration which increased with time (Fig. 11).

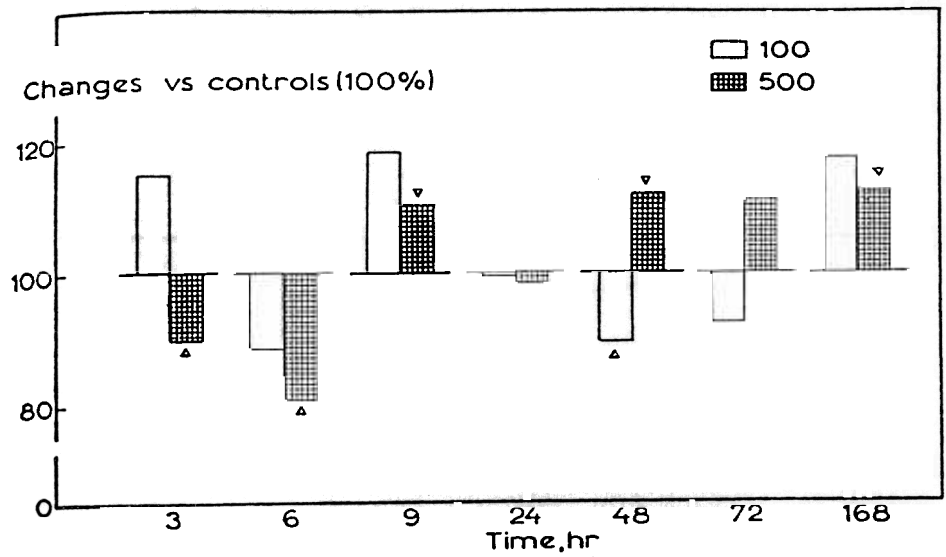


FIG. 9. Changes of intracellular K^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

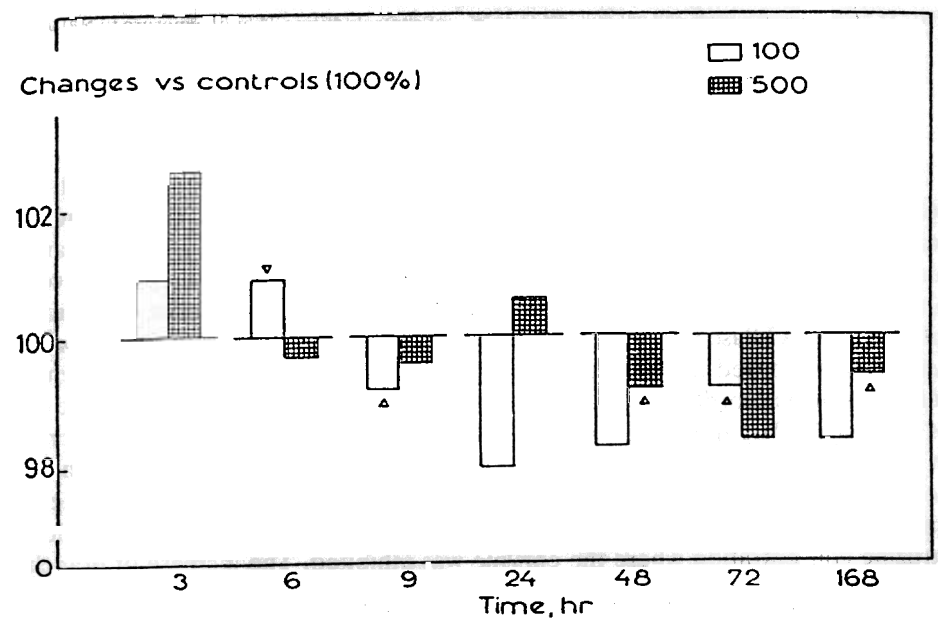


FIG. 10. Changes of muscle cell volume in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

The initial reduction of Na^+-K^+-ATPase specific activity was accompanied by a significant decrease in the level of plasma Na^+ and K^+. Likewise, Renfro *et al.* (1974) attributed the observed mercury-induced depression of Na^+ and K^+ transport in several osmoregulatory organs of different fresh and sea water teleosts to an inhibition of the Na^+, K^+-ATPase activity. On the other hand our observations seems to be at variance with the conclusions obtained by several other investigators. For example, Bouquegneau (1977) related elevated Na^+ and Cl^- levels in the plasma of sea water adapted eels, *Anguilla anguilla,* to an inhibition of the Na^+, K^+-ATPase activity of the gills. Lock *et al.* (1981) have also found a correlation between inhibition of the Na^+, K^+-ATPase in the gill of *Salmo gairdneri,* and its osmoregulation upon exposured to mercuric chloride and methylmercuric chloride but attributed this relation primarily to changes in the permeability characteristics of the gills for water. This may explain the absence of a correlation between the increase of the gill enzyme activity and plasma Na^+, K^+ levels of *Clarias gariepinus* exposed to both atrazine concentrations from the second day onwards.

From our previous discussion it appears therefore that Na^+, K^+-ATPase activation (500 μg/liter atrazine) or inhibition (100 μg/liter) may have value as an indicator of stress caused by atrazine. The disturbance of osmoregulation of the fish *Clarias garienpinus* exposed to atrazine at both concentrations affect also the level of the active cations at the muscle site.

The most important changes in the activity of gill Mg^{++} ATPase were represented by an activation of the enzyme from the 72th exposure hour onwards at both concentrations of atrazine. A similar simultaneous increase of both Na^+, K^+ and Mg^{++} ATPase was recorded by Watson and Beamish (1980) when they exposed the rainbow *Salmo gairdneri* to different zinc concentrations. They attributed their results to the zinc competition for Mg^{++} binding site in the gill epithelial membrane which might affect an increase in branchial permeability. Assuming that atrazine may act as Zn, the general augmentation of gill ATPase activities (Na^+, K^+ and Mg^{++}) could result from the diffusional loss of ions due to increased gill permeability. The ultimate reduction in ion concentration could, in turn, act as a signal for gill ATPase to increase the absorption rate of electrolytes.

The gill Na^+, K^+-ATPase of *Clarias gariepinus* was also extremely sensitive to DDT. The exposure of the fish to 500 μg/liter DDT produced a strong activation of gill Na^+, K^+-ATPase by exposure hr 3, then it decreased gradually with time to normal control level. Meanwhile, gill total protein remain constant throughout the whole experimental time which may indicate an activation of the present enzyme units. This result confirms similar reports by Khalifa (1989); and by Jowett *et al.* (1978), but are at odds with those of Hanke *et al.*, (1983); and Assem (1984) who reported striking inhibition by DDT of the Na^+, K^+-ATPase of the carp, *Cyprinus carpio.* The discrepancy may indicate that the fish, *Clarias gariepinus,* is less sensitive to DDT than the carp.

Of particular interest was the reduction of plasma Na^+ level by DDT treatment. This indicate that the fish's ability to maintain the sodium ion concentration at a con-

Gluth, G. and **Hanke, W.** (1983) The effect of temperature on physiological changes in carp, *Cyprinus carpio* L., induced by phenol. *Ecotoxicol. Environm. Safety* **7**, 373-389.

Hanke, W., Gulth, G., Bubel, H. and **Muller, R.** (1983) Physiological changes in carps induced by pollution. *Ecotox. Environ. Safety* **7**: 229-241.

Jampol, L.M. and **Epstein, F.H.** (1970) Sodium-potassium activated adenosine triphosphatase and osmotic regulation by fishes. *Am. J. Physiol.* **218**: 607-611.

Jowett, P.E., Rhead, M.M. and **Bayne, B.L.** (1978) In vitro changes in the activity of ATPases in the gills of *Carcinus maenas* exposed to various concentrations of p,p'-DDT. *Envir. Pollut.* **17**: 1-6.

———— (1981) In vivo changes in the activity of gill ATPase and haemolymph ions of *Carcinus maenas* exposed to p,p'-DDT and reduced salinities. *Comp. Biochem. Physiol.* **69C**: 399-402.

Khalifa, A. (1989) *Physiological responses of the fish Clarias lazera to some organic chemicals.* M.Sc. Thesis, Fac. Sc., Alexandria University, 185 p.

Kinter, W.B. and **Pritchard, J.B.** (1977) Altered permeability of cell membranes. In: *Handbook of Environmental Physiology - 1. Physical and Chemical Agents* (Edited by **Lee, D.H.K., Falk, H.L. Murphy, S.D.** and **Geiger, S.R.**), American Physiological Society, Washington, D.C., pp. 563-576.

Leadman, T.P., Cambell, R.D. and **Johnson, D.W.** (1974). Osmoregulatory responses to DDT and varying salinities in *Salmo gairdneri*. I. Gill Na^+ATPase. *Comp. Biochem. Physiol.* **49A**: 197-205.

Lock, R.A.C., Cruijsen, P.M.J.M. and **Van Overbeeke, A.P.** (1981) Effects of mercuric chloride on the osmoregulatory function of the gills in rainbow trout, *Salmo gairdneri,* Richardson. *Comp. Biochem. Physiol.* **68C**: 151-159.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and **Randall, R.J.** (1951) Protein measurement with the folin phenol reagent. *Biol. Chem.* **193**: 265-275.

Miller, D.S. and **Kinter, W.B.** (1977) DDT inhibits nutrients absorption and osmoregulatory function in *Fundulus heteroclitus.* In: *Physiological Responses of Marine Biota to Pollutants* (Edited by **Vernberg, F.J., Calabrese, A., Thurberg, F.P.** and **Vernberg, W.B.**), Academic Press, New York, pp. 63-74.

Neufield, G.J. and **Pritchard, J.B.** (1979a) Osmoregulation and gill Na^+-K^+-ATPase in the rock crab, *Cancer irroratus,* response to DDT. *Comp. Biochem. Physiol.* **62C**: 165-172.

———— (1979b) An assessment of DDT toxicity on osmoregulation and gill Na, K-ATPase activity in the blue crab. In: *Proceeding of the Symposium on Aquatic Toxicology* (Edited by **Marking, L.L.** and **Kimerle, R.A.**), American Society for Testing and Materials, pp. 23-34.

Renfro, J.L., Schmidt-Nielsen, B., Miller, D., Enos, D. and **Allen, J.** (1974) Methylmercury and inorganic mercury; uptake, distribution and effect of osmoregulatory mechanisms in fishes. In: *Pollution and Physiology of Marine Organisms* (Edited by **Vernberg, F.J.** and **Vernberg, W.B.**), Academic Press, New York, pp. 101-122.

Schmidt-Nelson, B., Renfro, J.L. and **Benos, D.** (1972) Estimation of extracellular space and intracellular ion concentration in osmoconformers, hypo- and hyper-osmoregulators. *Bull. Mt. Desert. Isl. Biol. Lab.* **12**: 99-104.

Watson, T.A. and **Beamish, F.W.H.** (1980) Effects of zinc on branchial ATPase activity in vivo in rainbow trout, *Salmo gairdneri. Comp. Biochem. Physiol.* **66C**: 77-82.

Watson, T.A. and **Beamish, F.W.H.** (1981) The effect of zinc on branchial adenosine triphosphatase enzymes in vitro from rainbow trout, *Salmo gairdneri. Comp. Biochem. Physiol.* **68C**: 167-173.

Zaugg, W.S. and **Mclain, L.R.** (1970) Adenosinetriphosphatase activity in gills of Salmonids: Seasonal variations and salt water influence in coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Phvsiol.* **35**: 587-596.